The Crystal Structure of the *Escherichia coli* MobA Protein Provides Insight into Molybdopterin Guanine Dinucleotide Biosynthesis

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Beamline(s): **X26C, X12B**

Introduction: The molybdenum cofactor (Moco) is found in a variety of enzymes present in all phyla, and comprises a family of related molecules containing molybdopterin (MPT), a tricyclic pyranopterin with a cis-dithiolene group as the invariant essential moiety. MPT biosynthesis involves a conserved pathway, but some organisms perform additional reactions that modify MPT. In *Escherichia coli*, the MobA protein links a guanosine 5'-phosphate to MPT forming molybdopterin guanine dinucleotide (MGD). This reaction requires GTP, $MgCl_2$, and the MPT form of the cofactor. The MobA monomer contains 194 amino acids and has a α/β architecture in which the N-terminal half of the molecule adopts a Rossman fold. The co-crystal structure of MobA and GTP reveals that the GTP binding site is located in the N-terminal half of the molecule. Conserved residues located primarily in three signature sequence motifs form crucial interactions with the bound nucleotide. The C-terminal half of the molecule, which contains another set of conserved residues, presumably is involved in the binding of MPT.

Methods and Materials: Crystals of MobA were obtained by vapor diffusion of an enzyme solution at a concentration of 12 mg/ml against a reservoir containing (2.5-3.0)M sodium acetate in 0.1M cacodylic acid, pH6.5. The crystals belong to the tetragonal space group I422 with a=124.1Å and c=67.0Å and contain one molecule in the asymmetric unit. The structure of MobA was solved by single isomorphous replacement and anomalous scattering using a Pt-derivative. The heavy atom derivative was prepared by soaking a crystal for 24 hours in mother liquor containing 2mM $K_2[PtCl_4]$. After transfer into a cryoprotectant containing 25% glycerol, the crystals were cryo-cooled and diffraction data were collected to 1.65Å(Native) and 2.7Å(Pt-derivative) resolution at beam lines X26C(Native) and X12B(Pt-derivative), respectively. Remote wavelength data collected at 1.033Å was chosen as heavy atom derivative data set. One major and three minor Pt-sites were detected by direct methods using SHELX. Phase refinement was performed with SHARP at 2.7Å resolution followed by solvent flattening and gradual phase extension to 1.7Å with SOLOMON. Subsequently, the resulting phases were used to auto-build the polypeptide chain using amplitudes to 1.65Å resolution with ARP after 5% of the data had been set aside to calculate the free R-factor. The model was refined with REFMAC and water molecules were added with ARP.

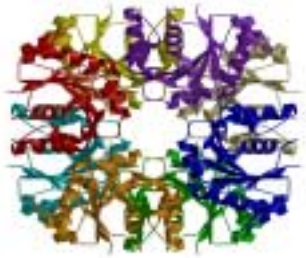

Figure 1. Oligomerization of MobA. Ribbon diagram of the MobA octamer viewed along the fourfold axis. Each color represents a different monomer. Four Zn ions located in the monomer-monomer interfaces are shown as gray spheres.